REARRANGEMENT OF THE T-CELL-RECEPTOR β-CHAIN GENE IN THE DIAGNOSIS OF LYMPHOPROLIFERATIVE DISORDERS

N. T. J. O'CONNOR* J. S. WAINSCOAT*
D. J. WEATHERALL* K. C. GATTER†
A. C. FELLER‡ P. ISAACSON§
D. JONES¶ K. LENNERT‡
G. PALLESEN|| A. RAMSEY§
H. STEIN** D. H. WRIGHT¶
D. Y. MASON†

*MRC Molecular Haematology Unit, University of Oxford, John Radcliffe Hospital, Oxford; †Department of Pathology, University of Oxford, John Radcliffe Hospital, Oxford; ‡Institute for Pathology, Kiel, West Germany; §Department of Histopathology, University College Hospital, London; ¶Department of Histopathology, University of Southampton; ||Institute of Pathology, University of Aarhus, Denmark; **Department of Histopathology, University of Berlin, West Germany*

Summary The arrangement of the T-cell-receptor and immunoglobulin genes has been analysed in 77 cases of lymphoproliferative disorder. All 6 T-cell leukaemias and 16 of 19 T-cell lymphomas showed rearrangement of the gene coding for the β chain of the T-cell receptor, associated in all cases with a germline arrangement of the immunoglobulin genes. All 36 B-cell leukaemias and all 16 B-cell lymphomas showed rearrangement of immunoglobulin genes; the T-cell-receptor gene was in the germline configuration in most of these cases but showed a rearranged pattern in 3 cases (2 chronic lymphatic leukaemias and 1 immunoblastic lymphoma). The combined use of T-cell-receptor and immunoglobulin gene probes promises to be a valuable means of identifying and classifying T-cell neoplasms.

Introduction

THE diagnosis of human lymphoproliferative disorders has been greatly facilitated by the introduction of monoclonal antibodies which recognise cell surface antigens. Immunohistological labelling of lymphoid tissue sections with these reagents not only permits the accurate immunological classification of malignant lymphomas[1] but may also enable benign disorders to be distinguished from malignant conditions.[2] Nevertheless a number of major diagnostic difficulties remain. Biopsy specimens of neoplastic lymphoid tissue often contain many reactive normal cells, which can obscure the immunohistological labelling reactions of the underlying malignant cells. Specimens containing large numbers of T cells also pose diagnostic problems, since in the absence of a marker for clonality (comparable to immunoglobulin [Ig] light-chain restriction seen in B-cell neoplasms), it may be impossible to decide whether these cells are neoplastic.

Recent reports have shown that gene mapping with DNA probes specific for Ig heavy and light chain genes may provide an alternative means of demonstrating the monoclonal B-cell origin of a leukaemia or lymphoma. This procedure involves demonstrating that one or more of these genes has undergone rearrangement in a clonal fashion. This approach has provided evidence for the B-cell origin of common acute lymphoblastic leukaemia and hairy-cell leukaemia.[3,4]

Until recently no comparable technique has been available to demonstrate monoclonality of T cells. The specificity of the T-cell response to foreign antigens depends on the T-cell receptor (TCR), which recognises and binds foreign antigen in the presence of major histocompatibility antigens. The TCR is a heterodimer consisting of two polypeptide chains, designated α and β, which are linked by a disulphide bond. The gene coding for the β chain of the TCR has now been cloned and shown to have a structure very similar to Ig light-chain genes.[5] This TCR β-chain gene undergoes rearrangement before transcription to messenger RNA (fig 1). Thus the demonstration of a rearrangement of this gene in proliferating lymphoid cells provides evidence for their T-cell origin. Both T-cell lines and leukaemias have been shown to rearrange the TCR β-chain gene, and this appears to occur early in T-cell development.[6,7]

In the present study, by analysing the TCR β-chain genes in 25 proven T-cell neoplasms of different histological types, and comparing the findings with those obtained for B-cell leukaemia and lymphoma, we have shown that the demonstration of rearrangements of the TCR β-chain genes is of practical use in the diagnosis of T-cell lymphomas.

Methods

Samples

Frozen tissue samples from cases of B and T lymphoma were obtained from material stored in the various histopathology departments involved in this study. The cases were classified histologically by examination of conventionally processed paraffin-embedded tissue and characterised as being of B or T origin with immunoenzymatic techniques using panels of monoclonal antibodies.[1]

Blood and bone-marrow samples from cases of leukaemia were obtained from the haematology department, John Radcliffe Hospital, and phenotyped by means of immuno-alkaline-phosphatase labelling of air-dried cell smears as described previously.[8] Blood was obtained from 36 healthy Caucasian volunteers as control samples.

DNA Analysis

DNA was extracted from blood or tissue samples by routine methods and digested with restriction endonucleases. The DNA fragments were subjected to electrophoresis in an 0·8% agarose gel and transferred to nitrocellulose filters by Southern blotting.[9] Filters were hybridised with specific ^{32}P-labelled plasmid probes, washed under appropriate conditions, and subjected to autoradiography. The three probes used in this study were an Ig heavy-chain joining region J_H probe (C76R51A), an Ig kappa-chain constant region C_k probe (pUCR17C_k), and a TCR β-chain probe (Jurkat β2).[5]

Immunoglobulin genes.—All DNA samples were digested with *Bst*I and with either *Hind*III or *Bgl*II before hybridisation with the J_H and C_k probes. All control samples showed identical restriction fragment patterns with the above enzymes and probes; no DNA polymorphisms were observed.

T-cell-receptor β-chain gene.—All DNA samples were digested with *Bst*I and *Hind*III and hybridised with the TCR β-chain probe. All control samples showed identical patterns with *Bst*I and (with one

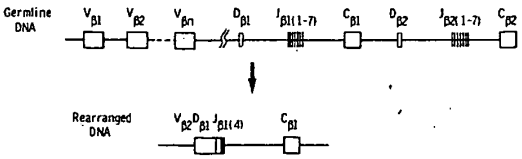

Fig 1—Assembly of the T-cell-receptor β-chain gene from separately encoded variable (V_β), diversity (D_β), joining (J_β), and constant (C_β) segments.

Messenger RNA is produced by processing of the primary transcript, V(D)J-C, from the rearranged gene segments.

TABLE I—IMMUNOGLOBULIN AND T-CELL RECEPTOR GENE ARRANGEMENTS IN LYMPHOPROLIFERATIVE DISORDERS

Diagnosis	Rearranged TCR, germline J_H	Germline TCR, germline J_H	Rearranged TCR, rearranged J_H	Germline TCR, rearranged J_H
T lymphoma	16	3	0	0
T leukaemia	6	0	0	0
B lymphoma	0	0	1	15
B leukaemia	0	0	2	34

exception) with *Hind*III. The single exception showed an additional variant band (13 Kb), which was subsequently shown to be inherited in a mendelian fashion. At least two different restriction enzymes were used to detect rearrangements, in order to exclude the possibility of abnormal patterns being caused by DNA polymorphism. No two T-cell neoplasms showed the same pattern of rearrangement. 3 cases of T-cell lymphoma which showed no evidence of TCR β-chain rearrangement after digestion with *Bst*I and *Hind*III were further studied after digestion with *Eco*RV and *Eco*RI.

Results

The results obtained from the study of DNA from 77 cases of lymphoproliferative disease are summarised in table I.

T-cell Neoplasia (Table II)

All 6 cases of T-cell leukaemia (2 acute lymphoblastic leukaemia and 4 chronic lymphocytic leukaemia [CLL]) showed rearrangement of the TCR β-chain gene but germline (non-rearranged) patterns with the J_H and C_k Ig probes. 19 cases of T-cell lymphoma were analysed, and 16 (85%) revealed rearrangement of the TCR β-chain gene, together with germline patterns with the J_H or C_k Ig gene probes. However, the remaining 3 cases showed a germline pattern for the TCR β-chain gene after digestion with four different enzymes. (*Bst*I, *Hind*III, *Eco*RI, and *Eco*RV) as well as germline configuration with both the J_H and C_k probes. Representative rearrangement patterns are shown in fig 2.

TABLE II—IMMUNOLOGICAL AND GENOTYPIC CLASSIFICATION OF 25 T-CELL NEOPLASMS

Morphological diagnosis	Monoclonal antibody studies*			Gene configuration†	
	T_3	T_4	T_8	TCR β chain	Ig heavy chain
T-cell leukaemia:					
1. T-CLL	+	−	+	R	G
2. T-CLL	+	+	−	R	G
3. T-CLL	+	−	−	R	G
4. T-CLL	+	+	−	R	G
5. T-ALL	−	−	+	R	G
6. T-ALL	+	+	+	R	G
T-cell lymphoma:					
1. Lymphoblastic	+	+	−	R	G
2. Lymphoblastic	+	−	−	R	G
3. Lymphoblastic	+	+	−	R	G
4. Peripheral	−	+	−	R	G
5. Peripheral	+	+	−	R	G
6. Peripheral	+	−	−	R	G
7. Peripheral	+	+	−	R	NA
8. Peripheral	+	−	−	R	G
9. Peripheral	+	+	−	R	G
10. Peripheral	+	−	−	R	G
11. Peripheral	+	−	+	R	G
12. Lennerts	+	+	−	R	G
13. Immunoblastic	+	+	−	R	G
14. Mycosis fungoides	+	+	−	R	G
15. Prolymphocytic	+	+	−	R	G
16. Prolymphocytic	+	+	−	R	G
17. Peripheral	+	+	−	G	G
18. Peripheral	+	+	+	G	G
19. Lymphoblastic	+	−	−	G	G

R = rearranged, G = germline (non-rearranged) configuration. NA = not available. *The T-cell phenotype was determined from analysis with a wide panel of monoclonal antibodies, the reactions shown being restricted to those of a pan-T marker (T_3), a T helper (T_4), and a T suppressor (T_8) marker. †See text for details of the restriction enzymes used for this analysis.

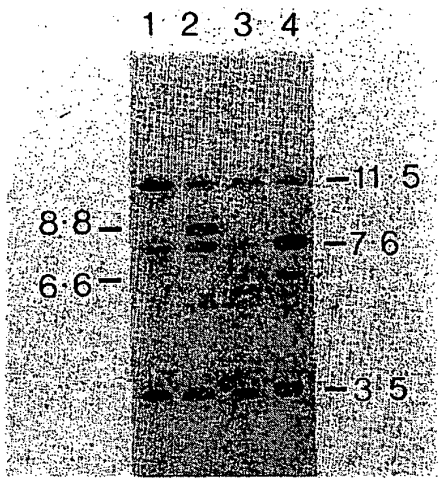

Fig 2—Autoradiograph of DNA digested with Hind III and then hybridised with the T-cell-receptor β-chain probe.

Track 1 contains DNA extracted from normal neutrophils; tracks 2, 3, and 4 contain DNA extracted from T-cell lymphomas. Tracks 2 and 4 both show additional bands indicating that a rearrangement has occurred, but track 3 gives a normal pattern (see text). The band sizes are shown in kilobases, the normal bands on the right and the abnormal bands on the left of the figure.

B-cell Neoplasia

36 cases of CLL and 16 cases of B-cell lymphoma of different histological types were studied. All cases showed rearrangement of the J_H Ig gene region as expected. In addition, all 52 cases were examined by means of hybridisation with the TCR β probe; 3 of the 52 (2 cases of B CLL and 1 case of immunoblastic B-cell lymphoma) showed a rearrangement pattern.

Discussion

The great majority of malignant lymphomas are believed to represent malignant proliferations arising from either B or T lymphoid cells. Evidence for monoclonality in B-cell lymphomas is usually based on the demonstration of light-chain restriction and, more recently, on the analysis of the arrangement of Ig genes. Before a B cell can produce immunoglobulin the Ig genes must undergo rearrangement, and this occurs early in B-cell ontogeny. In a monoclonal proliferation of B cells the Ig genes which have rearranged will all have undergone the same rearrangement(s), and this can be readily detected by gene analysis. However, no Ig gene rearrangements are detected in polyclonal B-cell proliferations, since no single rearranged pattern occurs at a sufficiently high frequency. The demonstration of an Ig gene rearrangement thus provides evidence that a lympho-proliferative disorder is both monoclonal and B-cell derived, and this is helpful in the distinction between a malignant and a polyclonal B-cell expansion.[10,11]

The isolation of the gene coding for the β chain of the TCR has allowed us to examine whether DNA analysis with this probe may be of equal value in the objective diagnosis of T-cell lymphomas. All 6 cases of T-cell leukaemia showed a rearranged pattern of their TCR β-chain genes, while showing a germline pattern with the Ig gene probes. Of 19 T-cell lymphomas, 16 showed rearrangement of the TCR β-chain genes, whereas the Ig genes all showed a germline pattern. Thus, rearrangement of the β-chain TCR gene accompanied by germline Ig genes provides good evidence that a tumour is T-cell derived.

3 cases of lymphoma typed as being of T-cell origin by means of monoclonal antibody studies did not show TCR β-chain rearrangement. Since rearrangement of the TCR β-chain gene occurs early in T-cell development (at the cortical thymocyte stage[12]), immaturity of the malignant T cell is not a probable explanation for the lack of TCR rearrangement in these cases. Furthermore, it is unlikely that rearrangement was masked by the presence of numerous reactive cells, since dilution experiments have shown that a monoclonal population of T cells can be detected when their DNA represents only 5% of that present in a sample (unpublished results).

This leaves at least two possible alternative explanations for the three T-cell lymphomas which showed no detectable rearrangement of the TCR β-chain gene. First, it is conceivable that the phenotypic typing in these biopsy specimens was misleading and that the T-cell proliferation was a polyclonal response to a neoplasm of non-T-cell origin. However, if this is the case the cell of origin in these particular tumours is unknown, since none of the cases showed a rearrangement of the Ig genes (which would have indicated a B-cell neoplasm). Alternatively, deletion (rather than rearrangement) of the gene may have occurred, and the germline pattern observed on gene analysis with the TCR probe would therefore be due to the presence of reactive (non-malignant) cells in the biopsy sample.

2 cases of B-cell CLL and 1 case of B-cell immunoblastic lymphoma appeared to show dual rearrangement of both Ig heavy-chain and TCR β-chain genes. This phenomenon has not been reported previously, but it may be analogous to the rearrangement of Ig heavy-chain genes which has been reported in T-cell lines.[3] It may also be of relevance that Rovigatti et al[13] have reported 2 cases of acute myeloblastic leukaemia in which Ig gene rearrangement could be shown. This suggests that aberrant TCR and Ig gene rearrangement may occasionally be encountered in cells which have no functional requirement for this process.[3,6]

Our results suggest that neoplasms showing TCR β-chain gene rearrangement associated with germline Ig genes are T-cell derived. Other rearrangement patterns may be less informative, although rearrangement of the Ig light-chain gene appears specific to tumours of B-cell origin.[3] If our preliminary conclusions are supported in the more extensive studies now in progress, a novel and powerful means of objectively diagnosing T-cell neoplasia will be available for use in clinical practice.

We thank Dr Tak Mak for the T-cell receptor Jurkat β2 probe, Dr T. A. R. Rabbitts for the J_H and C_k probes, and Lisa Clarke for technical assistance. N. T. J. O'C. is in receipt of a Medical Research Council training fellowship. This work was supported by the Medical Research Council, the Leukaemia Research Fund, the Wellcome Trust, and the Cancer Research Campaign.

Correspondence should be addressed to D. Y. M., Haematology Department, John Radcliffe Hospital, Oxford OX3 9DU.

References at foot of next column

LONG-TERM FOLLOW-UP OF PATIENTS WHO UNDERWENT UNILATERAL NEPHRECTOMY IN CHILDHOOD

PIERRE ROBITAILLE JEAN-GUY MONGEAU
LOUISE LORTIE PATRICK SINNASSAMY

Department of Paediatrics, Nephrology Division, Hôpital Sainte-Justine, University of Montreal, Montreal, Quebec, Canada

Summary The long-term damaging potential of remnant nephron hyperperfusion was investigated in patients who had undergone unilateral nephrectomy in childhood. 27 such patients were examined after a mean of 23·3 years postnephrectomy. The average creatinine clearance was 83·9±16·5 ml/min/1·73 m^2 or 74·3% of that in healthy controls with two kidneys; it was a value similar to that reported 3 to 6 months postnephrectomy in kidney donors. Age at the time of nephrectomy, duration of follow-up, or sex had no influence on the residual creatinine clearance. None of these patients had clinically important hypertension or proteinuria. Since so little evidence of kidney damage could be documented after such a long observation period, hyperperfusion would seem to be seldom of clinical importance in man unless other factors were present.

Introduction

SUBSTANTIAL renal mass reduction in animals leads to progressive azotaemia, proteinuria, and glomerulosclerosis.[1-3] After extensive renal ablation, single nephron glomerular filtration rate (GFR) in the functioning nephrons increases because of a rise in glomerular capillary hydraulic pressure and an augmented glomerular capillary plasma flow rate.[3] These physiological changes lead to glomerular lesions, including endothelial and epithelial damage and an increase in mesangial matrix,[2,3] and eventually glomerulosclerosis.[2] Several reports have suggested that the same sequence of events operates in man to produce proteinuria, hypertension, and even renal failure in patients with renal agenesis[4,5] and in

N. T. J. O'CONNOR AND OTHERS: REFERENCES

1. Stein H, Lennert K, Feller A, Mason DY. Immunohistological analysis of human lymphomas. In: Klein G, Weinhouse S, eds. Advances in cancer research. Orlando: Academic Press, 1984: 67–141.
2. Gatter KC, Falini B, Mason DY. The use of monoclonal antibodies in histopathological diagnosis In: Anthony PP, MacSween RNM, eds. Recent advances in histopathology. Edinburgh; Churchill Livingston, 1984: 35–68.
3. Korsmeyer SJ, Arnold A, Bakshi A, et al. Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphoblastic leukaemias of T-cell and B-cell precursor origins. *J Clin Invest* 1983; 71: 301–13.
4. Korsmeyer SJ, Greene WC, Cossman J, et al. Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in hairy cell leukaemia. *Proc Natl Acad Sci USA* 1983; 80: 4522–26.
5. Yanagi Y, Yoshikai Y, Leggett K, Clark SP, Aleksander I, Mak TW. A human T cell-specific cDNA clone encodes a protein having extensive homology to immunoglobulin chains. *Nature* 1984; 308: 145–49.
6. Sims JE, Tunnacliffe A, Smith WJ, Rabbitts TH. Complexity of human T-cell antigen receptor β-chain constant- and variable-region genes. *Nature* 1984; 312: 541–45.
7. Minden M, Toyonaga B, Ha Y, Yanagi Y, Chin B, Gelfand E, Mak TW. Somatic rearrangement of the T-cell antigen receptor gene in human T-cell malignancies. *Proc Natl Acad Sci USA* 1985; 82: 1224–28.
8. Moir DJ, Ghosh AK, Abdulaziz Z, Knight PM, Mason DY. Immunoenzymatic staining of haematological samples with monoclonal antibodies. *Br J Haematol* 1983; 55: 395–410.
9. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975; 98: 503–17.
10. Arnold A, Cossman J, Bakshi A, Jaffe ES, Waldmann TA, Korsmeyer SJ. Immunoglobulin gene rearrangements as unique clonal markers in human lymphoid neoplasms. *N Engl J Med* 1983; 309: 1593–99.
11. Cleary ML, Chao J, Warnke R, Sklar J. Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. *Proc Natl Acad Sci USA* 1984; 81: 593–97.
12. Toyonaga B, Yanagi Y, Suciu-Foca N, Minden M, Mak TW. Rearrangement of T cell receptor gene YT35 from thymic leukaemic T-cell lines and functional T-cell clones. *Nature* 1984; 311: 385–87.
13. Rovigatti U, Mirro J, Kitchingman G, Dahl G, Ochs J, Murphy S, Stass S. Heavy chain immunoglobulin gene rearrangement in acute nonlymphocytic leukaemia. *Blood* 1984; 63: 1023–27.